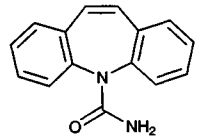
Carbamazepine



Molecular formula: $C_{15}H_{12}N_2O$
Molecular weight: 236.27
CAS Registry No.: 298-46-4
Merck Index: 1826
Lednicer No.: 1 403

SAMPLE

Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 30 mg/L IS in water + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 2 30 μm Permaphase ETH (DuPont)
Column: 250 × 4.6 Zorbax Stable-Bond CN
Mobile phase: MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
Column temperature: 50
Flow rate: 1.2
Injection volume: 50
Detector: UV 214

CHROMATOGRAM

Retention time: 11.5
Internal standard: cyheptamide (14)
Limit of detection: 15-30 ng/mL

OTHER SUBSTANCES

Extracted: carbamazepine diol, carbamazepine epoxide, lamotrigine, 5-(p-hydroxyphenyl)-5-phenylhydantoin, phenytoin
Simultaneous: acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

KEY WORDS

serum; SPE

REFERENCE

Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

SAMPLE

Matrix: blood
Sample preparation: Mix 500 μL plasma with 850 μL MeCN, vortex, centrifuge at 1500 g for 5 min. Inject an aliquot.

HPLC VARIABLES
Column: 33 × 4.6 1.5 μm Kovasil MS C14 (CU Chemie Uetikon, Switzerland)
Mobile phase: MeCN:20 mM pH 7.0 potassium phosphate buffer 7:93
Column temperature: 60
Flow rate: 1.2
Injection volume: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 2.27
Limit of quantitation: 4 μM

OTHER SUBSTANCES
Simultaneous: metabolites, clobazam, desmethylclobazam, ethosuximide, felbamate, pheneturide, (±)-5-(p-hydroxyphenyl)-5-phenylhydantoin, 5-(m-hydroxyphenyl)-5-phenylhydantoin phenobarbital, phenylethylmalonamide, phenytoin, primidone
Noninterfering: vigabatrin, valproic acid

KEY WORDS
plasma

REFERENCE
Chollet,D.; Castella,E.; Combe,P.; Arnera,V. High-performance liquid chromatographic method for the monitoring of carbamazepine and its active metabolite, carbamazepine-10,11-epoxide, in human plasma, *J.Chromatogr.B*, **1996**, *683*, 237–243.

SAMPLE
Matrix: blood
Sample preparation: Mix 500 μL plasma with 500 μL MeCN and 2 μg IS for 30 s, centrifuge at 2700 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:MeOH:10 mM pH 7.4 phosphate buffer 15:35:50
Column temperature: 25
Flow rate: 1
Detector: UV 219

CHROMATOGRAM
Internal standard: 2-hydroxy-2-ethyl-2-phenylacetamide
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: clonazepam, ethosuximide, D,L-2-hydroxy-2-ethyl-2-phenylpropionamide (HEPP), phenobarbital, phenytoin, primidone

KEY WORDS
rat; plasma

REFERENCE
Martínez de Muñoz,D.; Arenas,R.; Chávez González,O. Liquid chromatographic assay in plasma of one of the members of a new series of anticonvulsants: D,L-3-hydroxy-3-ethyl-3-phenylpropionamide, *J.Chromatogr.B*, **1996**, *678*, 377–383.

SAMPLE
Matrix: blood

Sample preparation: Add 200 μL 2 μg/mL thymol in MeCN to 200 μL serum, vortex for 10 s, centrifuge at 7000 g for 5 min, inject 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Resolve C18-5 (Waters)
Mobile phase: MeCN:isopropanol:50 mM pH 3.0 phosphate buffer 25:15:60
Column temperature: 30
Flow rate: 0.7
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 9.0
Internal standard: thymol (18.5)

OTHER SUBSTANCES
Extracted: ethosuximide, primidone, phenobarbital, phenytoin, valproic acid

KEY WORDS
human; plasma

REFERENCE
Kondo,K.; Nakamura,M.; Nishioka,R.; Kawai,S. Direct method of determination of valproic acid in serum by high performance liquid chromatography, *Anal.Sci.*, **1985**, *1*, 385–387.

SAMPLE
Matrix: blood
Sample preparation: Dilute 20 μL serum with 100 μL pH 3.7 phosphate buffer, shake vigorously for 10 s, add to a 45 μL PTFE column packed with 50 μm ODS-silica (Asahi Chemicals, Tokyo) (Extrashot-ODS device), wash with 100 μL water, elute with 130 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 7 μm Hibar LiChrosorb RP-18
Mobile phase: MeCN:MeOH:pH 4.4 potassium phosphate buffer 14:21:65
Flow rate: 1
Injection volume: 100
Detector: UV 210

CHROMATOGRAM
Retention time: 18.7

OTHER SUBSTANCES
Extracted: phenobarbital, phenytoin

KEY WORDS
SPE

REFERENCE
Kouno,Y.; Ishikura,C.; Homma,M.; Oka,K. Extrashot-ODS, a syringe-type minicolumn sample injector for a reversed-phase high-performance liquid chromatographic column. Application to antiepileptics in human sera, *J.Chromatogr.B*, **1997**, *695*, 349–353.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 40 μg/mL 5-ethyl-5-p-tolylbarbituric acid and 3 mL dichloromethane to 100 μL plasma, vortex for 2 min, centrifuge at 1200 g for 5 min, evap-

orate the organic phase under a gentle nitrogen stream in a water bath at 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-ODS C18
Mobile phase: MeCN:water 30:70
Flow rate: 0.4
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 9.7
Internal standard: 5-ethyl-5-p-tolylbarbituric acid (8.8)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rat

REFERENCE
Tanaka,E. Simultaneous determination of carbamazepine and its metabolites in plasma from carbon tetrachloride-intoxicated rats using a new reversed-phase chromatographic column of 2-μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *688*, 155–160.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Inject a 5-20 μl aliquot onto the column with mobile phase A or B. Urine. Inject a 20 μL aliquot onto the column with mobile phase C.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeCN:20 mM pH 6.9 phosphate buffer 20:80 (A) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer from 5:95 to 20:80 over 2 min, to 25:75 over 2 min, to 30:70 over 4 min, to 50:50 over 2 min, maintain at 50:50 for 10 min (B) or Gradient. MeCN:20 mM pH 6.9 phosphate buffer 14:86 for 5 min, to 25:75 over 1 min, to 30:70 over 2 min, to 50:50 over 3 min, maintain at 50:50 for 6 min (C)
Flow rate: 1
Injection volume: 5 (A, C), 20 (B)
Detector: UV 254 (serum); UV 230 (urine)

CHROMATOGRAM
Retention time: 7.61 (serum, A), 15.5 (serum, B), 17.5 (urine,C)
Limit of detection: 3 ng (urine)

OTHER SUBSTANCES
Simultaneous: acetaminophen (B), barbital (B), phenobarbital (B,C), phenytoin (B,C), primidone (B,C), sulfapyridine (A,B)
Also analyzed: metabolites

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 213.4

CHROMATOGRAM

Retention time: 15.763

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 Zorbax SB C18

Mobile phase: Gradient. MeCN:20 mM pH 7.5 phosphate buffer from 30:70 to 40:60 over 10 min. (Mobile phase was contained 0.1% triethylamine.)

Injection volume: 10

Detector: UV 280

CHROMATOGRAM

Retention time: 7.3

OTHER SUBSTANCES

Simultaneous: omeprazole

REFERENCE

Sarich,T.; Kalhorn,T.; Magee,S.; Al-sayegh,F.; Adams,S.; Slattery,J.; Goldstein,J.; Nelson,S.; Wright,J. The effect of omeprazole pretreatment on acetaminophen metabolism in rapid and slow metabolizers of S-mephenytoin, *Clin.Pharmacol.Ther.*, **1997**, *62*, 21–28.

SAMPLE

Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco LC-8
Mobile phase: MeOH:100 mM K_3PO_4 45:55 containing 100 μL/L triethylamine

OTHER SUBSTANCES
Simultaneous: metabolites

REFERENCE
Cohen,H.; Howland,M.A.; Luciano,D.J.; Rubin,R.N.; Kutt,H.; Hoffman,R.S.; Leung,L.K.H.; Devinsky,O.; Goldfrank,L.R. Feasibility and pharmacokinetics of carbamazepine oral loading doses, *Am.J.Health-Syst.Pharm.*, **1998**, *55*, 1134–1140.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 ODS (Hitachi)
Mobile phase: MeCN:50 mM phosphoric acid 40:60adjusted to pH 5.5 with NaOH
Column temperature: 55
Flow rate: 0.6
Injection volume: 20
Detector: UV 285

OTHER SUBSTANCES
Also analyzed: fenbufen, indomethacin, ketoprofen, α.-naphthoquinone, naproxen, tolmetin

REFERENCE
Sugawara,M.; Takekuma,Y; Yamada,H.; Kobayashi,M.; Iseki,K.; Miyazaki,K. A general approach for the prediction of the intestinal absorption of drugs: regression analysis using the physicochemical properties and drug-membrane electrostatic interactions, *J.Pharm.Sci.*, **1998**, *87*, 960–966.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.74

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

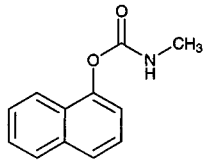
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

Carbaryl



Molecular formula: $C_{12}H_{11}NO_2$
Molecular weight: 201.22
CAS Registry No.: 63-25-2
Merck Index: 1831

SAMPLE

Matrix: beverages
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 4 mL MeOH and 5 mL water. Pass 60 mL juice through the cartridge, wash with 5 mL MeCN:water 25:75, elute with 2 mL MeCN:water 75:25, inject a 20-50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultremex
Mobile phase: MeCN:MeOH:water 15:40:45
Flow rate: 1
Injection volume: 20-50
Detector: UV 224

CHROMATOGRAM

Retention time: 4
Limit of detection: 5 ppb

KEY WORDS

fruit juice; SPE

REFERENCE

Bushway,R.J. High-performance liquid chromatographic determination of carbaryl in fruit juices, *J.Chromatogr.*, **1988**, *457*, 437–441.

SAMPLE

Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 12.89
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize tissue with an equal volume of water, treat with a saturated solution of calcium chloride, let stand overnight, filter. Extract filtrate, blood, or other body fluid with an equal volume of ether. Adjust pH of aqueous layer to 2 with 2 M HCl, extract with an equal volume of ether. Combine the ether layers, evaporate to dryness, reconstitute in a suitable solvent, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax cyano
Mobile phase: Iso-octane:ethyl acetate 80:20
Flow rate: 1
Injection volume: 20
Detector: RI

CHROMATOGRAM

Retention time: 8.70
Limit of detection: 100 ng

OTHER SUBSTANCES

Extracted: methyl parathion, dichlorvos, monocrotophos, quinalphos, malathion, phosphamidon, propoxur (baygon)

KEY WORDS

liver; lung

REFERENCE

Sharma,V.K.; Jadhav,R.K.; Rao,G.J.; Saraf,A.K.; Chandra,H. High performance liquid chromatographic method for the analysis of organophosphorus and carbamate pesticides, *Forensic Sci.Int.*, **1990**, *48*, 21–25.

SAMPLE

Matrix: blood, urine

Sample preparation: Evaporate 25 μL of a 6.5 μg/mL solution of napropamide into a tube, add 250 μL whole blood, plasma, or urine, add 250 μL water, wait for 5-10 min, add 2.5 mL ethyl acetate, shake for 10 min, centrifuge at 1200 g for 8 min. Remove 2 mL of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 250 μL EtOH:MeCN:130 mM pH 6.2 phosphate buffer 50:20:30, inject a 50-100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C8 (Alltech)
Mobile phase: MeCN:130 mM pH 6.2 phosphate buffer 40:60
Flow rate: 1.42
Injection volume: 50-100
Detector: F ex 285 em 340 (cut-off filter)

CHROMATOGRAM
Retention time: 10.6
Internal standard: napropamide (30)
Limit of quantitation: 40 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 1-naphthol

KEY WORDS
whole blood; plasma

REFERENCE
DeBerardinis,M.,Jr.; Wargin,W.A. High-performance liquid chromatographic determination of carbaryl and 1-naphthol in biological fluids, *J.Chromatogr.*, **1982**, *246*, 89–94.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 17.968

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: food

Sample preparation: 30 g Rice + 50 mL MeOH, let stand for 48 h with occasional manual shaking, inject a 10 μL aliquot of the MeOH layer. Alternatively, remove a 1 mL aliquot of the MeOH layer and evaporate it to near dryness under a stream of nitrogen, add 1 mL hexane, shake, repeat extraction. Combine the hexane layers and add them to a Sep-Pak Florisil SPE cartridge, elute with 3 mL acetone:hexane 40:60. Combine the eluates and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL MeOH, inject a 10 μL aliquot. (Recovery is 97% with direct injection or 30% with sample clean-up.)

HPLC VARIABLES

Guard column: Guard-Pak (Waters)

Column: 250 × 3.9 Nova-Pak C18

Mobile phase: Gradient. MeCN:water from 40:60 to 70:30 over 12 min or Isocratic (1) MeCN:water 60:40 or Isocratic (2) MeCN:water 40:60

Flow rate: 1

Injection volume: 10

Detector: UV 225

CHROMATOGRAM

Retention time: 4 (gradient), 2 (isocratic 1), 7 (isocratic 2)

Limit of detection: 50 ng/g (without clean-up)

OTHER SUBSTANCES

Extracted: methacrifos, fenitrothion, etrimfos, chlorpyrifos-methyl, pirimphos-methyl (UV 247) (with gradient or isocratic 1)

KEY WORDS

rice; SPE

REFERENCE

Brayan,J.G.; Haddad,P.R.; Sharp,G.J.; Dilli,S.; Desmarchelier,J.M. Determination of organophosphate pesticides and carbaryl on paddy rice by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *447*, 249–255.

SAMPLE

Matrix: food

Sample preparation: Prepare a column by adding 500 mg silanized Celite 545 to a 22 mm ID column, add 5 g Nuchar S-N:silanized Celite 545 20:80, wash with 50 mL MeCN:toluene 75:25, do not allow to go dry. Homogenize (Polytron) 150 g sample with 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter, remove a portion equivalent to 100 g sample, add water so that there is 100 mL water in the flask, concentrate to 75 mL under reduced pressure at 35°, add 15 g NaCl, add 75 mL MeCN, shake for 30 s, extract the aqueous layer with 50 mL MeCN for 20 s. Combine the organic layers and add them to 25 mL 20% NaCl, shake, discard the aqueous layer, wash the MeCN layer with 100 mL petroleum ether, extract the petroleum ether layer with 10 mL MeCN. Combine the MeCN layers and add 50 mL 2% NaCl, extract with 100 mL dichloromethane, extract twice with 25 mL portions of dichloromethane, filter the dichloromethane layers through 5 g anhydrous sodium sulfate, evaporate the dichloromethane extracts to dryness under reduced pressure, immediately reconstitute with 10 mL dichloromethane, add to the column, elute at 5 mL/min, rinse flask with 10 mL dichloromethane, add the rinse to the column, elute with 125 mL MeCN:toluene 75:25, collect all the effluent from the column and evaporate it just to dryness under reduced pressure, reconstitute with 5 mL MeOH, filter (5 μm), inject an aliquot. (Silanize Celite 545 by boiling 150 g Celite 545 in 1 L HCl:water 50:50 for 10 min, cool, filter, wash with water until the filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry, heat to 120°, cool

in desiccator, add 3 mL dichlorodimethylsilane, mix well, let stand at room temperature for 4 h, add 500 mL MeOH, mix, let stand for 15 min, filter, wash with isopropanol until neutral, air dry, dry at 105° for 2 h, cool in desiccator. Test for total silanization by placing 1 g in 20 mL toluene saturated with methyl red. Silanized Celite should appear yellow. Silanized Celite should also float on water. If silanization is not complete, repeat process. Boil 100 g Nuchar S-N with 700 mL HCl for 1 h, add 700 mL water, boil for 30 min, cool, filter, wash with water until the filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry, dry at 120° for 4 h, cool in desiccator.) (J. Assoc. Off. Anal. Chem. 1985, 68, 726).

HPLC VARIABLES
Guard column: 20 × 2 30-40 μm Perisorb RP-8
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 20:80 to 70:30 over 25 min, re-equilibrate at initial conditions for 10 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 20
Detector: E, ESA Model 5100A, Model 5010 dual analytical cell, detector 1 + 0.20 V, detector 2 +0.60 V (monitored), Model 5020 guard cell in NaOH stream at +0.70 V, following post-column reaction. The column effluent mixed with 100 mM NaOH pumped at 0.5 ± 0.02 mL/min and the mixture flowed through a 3 m × 0.48 mm ID stainless-steel coil at 100° to the detector.

CHROMATOGRAM
Retention time: 15
Limit of detection: 0.25 ng
Limit of quantitation: 10 ppb

OTHER SUBSTANCES
Extracted: bufencarb, carbofuran, 3-hydroxycarbofuran, isoprocarb, methiocarb

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KEY WORDS
post-column reaction; apples; cabbages; grapes; tomatoes

REFERENCE
Krause,R.T. High-performance liquid chromatographic determination of aryl N-methylcarbamate residues using post-column hydrolysis electrochemical detection, *J.Chromatogr.*, **1988**, *442*, 333–343.

SAMPLE
Matrix: fruit
Sample preparation: Blend 50 g chopped grapes with 100 mL MeCN, 25 mL water, and 10 g Celite for 15 min, filter (fritted glass). Dilute the filtrate with MeCN:water 50:50, evaporate a 10 mL aliquot to dryness under reduced pressure, reconstitute with 3 mL MeOH, filter (0.2 μm Nylon), wash the filter with 2 mL MeOH, evaporate the filtrate to 1 mL, dilute with 5 mL THF, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5 amino
Mobile phase: Gradient. THF:10 mM tetramethylammonium hydrogen sulfate:50 mM pH 6.7 acetate buffer 99.8:0.2:0 for 10 min, to 98.9:0.1:1 over 1 min, maintain at 98.9:0.1: for 9 min
Flow rate: 1
Injection volume: 20
Detector: F ex 280 em 333

CHROMATOGRAM
Retention time: 3.80

Limit of detection: 20 ng/g
Limit of quantitation: 70 ng/g

OTHER SUBSTANCES

Extracted: aminocarb (F ex 255 em 367), carbendazim (F ex 285 em 318), fuberidazol (F ex 310 em 345), 1-naphthylacetamide (F ex 282 em 340)

KEY WORDS

grapes

REFERENCE

García Sńchez,F.; Navas Díaz,A.; García Pareja,A. Normal phase liquid chromatography on amino-bonded-phase column of fluorescence detected pesticides, *J.Liq.Chromatogr.*, **1995**, *18*, 4543–2558.

SAMPLE

Matrix: fruit, grain, vegetables

Sample preparation: Homogenize (Polytron) 150 g high moisture sample and 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter (paper) under vacuum, remove portion of filtrate equal to 100 g sample and make up to 100 mL with water. Homogenize (Polytron) 75 g low moisture sample and 300 mL MeOH at half speed for 30 s and full speed for 1 min, filter (paper) under vacuum, remove portion of filtrate equal to 50 g sample and make up to 100 mL with water. Concentrate samples to 75 mL under reduced pressure at 35°, add 15 g NaCl, add 75 mL MeCN, shake for 30 s, let stand for 5 min. Remove the aqueous phase and add it to 50 mL MeCN, shake for 20 s, let layers separate, discard the aqueous layer. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, extract petroleum ether layer with 10 mL MeCN. Combine the MeCN layers and add them to 50 mL 2% NaCl, extract with 100 mL dichloromethane, extract twice with 25 mL portions of dichloromethane. Combine the dichloromethane layers and pass them through a 22 mm i.d. column containing 5 g anhydrous sodium sulfate. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute in 10 mL dichloromethane, add to the charcoal column, rinse flask with 10 mL dichloromethane, rinse flask with 25 mL MeCN:toluene 75:25. Evaporate the eluate to dryness under reduced pressure at 35°, reconstitute with 5 mL MeOH, filter (5 μm), inject a 10 μL aliquot (J. Assoc. Off.Anal. Chem. 1980, 63, 1114). (Charcoal column was 5 g silanized Celite 545: Nuchar S-N 4:1 on top of 0.5 g silanized Celite 545 in a 300 × 22 glass column, wash with 50 mL MeCN:toluene 75:25, do not allow to go dry. Prepare silanized Celite 545 as follows. Boil 150 g Celite 545 in 1 L 6 M HCl with stirring for 10 min, cool, filter, wash with water until filtrate is neutral, wash with 500 mL MeOH, wash with 500 mL dichloromethane, air dry in hood, heat to 120° in a flask, cool in a desiccator, add 3 mL dichlorodimethylsilane, mix well, let stand at room temperature for 4 h, add 500 mL MeOH, mix, let stand for 15 min, filter, wash with isopropanol until neutral, air dry in hood, dry at 105° for 2 h, cool in desiccator, store in stoppered container. Totally silanized Celite should float on water and appear yellow (not pink) in toluene saturated with methyl red (J. Assoc. Off.Anal. Chem. 1980, 63, 1114).)

HPLC VARIABLES

Guard column: 70 × 2.1 25-37 μm Co-Pell ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 12:88 to 70:30 over 30 min, 100:0 for 5 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: F ex 288 em 330 following post-column reaction. The column effluent mixed with 200 mM NaOH pumped at 0.5 mL/min and flowed through a 3 m × 0.48 mm stainless steel column to the detector.

CHROMATOGRAM

Retention time: 21

Limit of quantitation: 20 ppb

OTHER SUBSTANCES
Extracted: carbofuran, napropamide, phosalone, piperonyl butoxide

KEY WORDS
post-column reaction; pears; green beans

REFERENCE
Krause,R.T.; August,E.M. Applicability of a carbamate insecticide multiresidue method for determining additional types of pesticides in fruits and vegetables, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 234–240.

SAMPLE
Matrix: fruit, vegetables

Sample preparation: Homogenize (Omni-Mixer) 100 g chopped sample with 250 mL MeOH at half-speed for 5 min, filter (Whatman No. 1 PS paper), make up filtrate to 500 mL with MeOH. Remove 100 mL filtrate and add it to 125 mL 4% aqueous sodium sulfate, shake well, extract mixture with 75, 50, and 50 mL portions of dichloromethane with 30 s shaking each time, drain organic layers through anhydrous sodium sulfate. Combine the organic layers and evaporate them to 1 mL under reduced pressure at 30°, transfer residue to a tube with two 2 mL rinses of dichloromethane:cyclohexane 50:50, make volume up to 10 mL with dichloromethane:cyclohexane 50:50, filter (0.45 μm), add 5 mL to a 600 × 25 tube containing 60 g 200-400 mesh BioBeads SX-3 resin (Analytical Bio-Chemistry Laboratories), pump through at 5 mL/min with dichloromethane:cyclohexane 50:50 mobile phase, discard mobile phase for 24 min, collect fraction containing the compound for 12 min, evaporate under reduced pressure at 30° to low volume, add 15 mL MeOH, evaporate to about 1 mL, filter (0.45 μm), inject a 20 μL aliquot. Alternatively, run output from BioBeads column through a column containing 0.5 g of a mixture of Nuchar S-N(Fisher):Celite 545 1:4, at the end of the chromatography elute this column with 10 mL MeCN:toluene 75:25, evaporate the eluate under reduced pressure at 30° to low volume, add 15 mL MeOH, evaporate to about 1 mL, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.6 Pellicular ODS (Whatman)

Column: 250 × 4.6 5 μm Apex ODS (Jones Chromatography)

Mobile phase: Gradient. MeOH:water from 10:90 to 90:10 over 23 min, to 10:90 over 4 min, re-equilibrate at 10:90 for 10 min

Flow rate: 1

Injection volume: 20

Detector: F ex 340 em 455, following post-column derivatization. The column effluent is mixed with 200 mM NaOH at 0.8 mL/min and the mixture flows through a 1 mL coil at 95° and is mixed with 500 mg/L o-phthalaldehyde and 1 mL/L 2-mercaptoethanol in 50 mM sodium tetraborate pumped at 0.8 mL/min. The mixture flows through a 0.5 mL coil at ambient temperature to the detector.

CHROMATOGRAM
Retention time: 25

Limit of detection: 5-10 ppb

OTHER SUBSTANCES
Extracted: oxamyl, methomyl, aldicarb, propoxur, carbofuran, methiocarb

KEY WORDS
apples; broccoli; cabbage; cauliflower; potatoes; post-column reaction; derivatization

REFERENCE

Chaput,D. Simplified multiresidue method for liquid chromatographic determination of N-methyl carbamate insecticides in fruits and vegetables, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 542–546.

SAMPLE

Matrix: soil

Sample preparation: 100 g Soil + 100 mL water, blend, add 125 mL MeOH, blend for 5 min, centrifuge for 15 min, let stand for 10 min. Remove the supernatant and extract the residue with 50 mL MeOH. Combine the supernatants and extract them three times with 100 mL portions of dichloromethane, dry the organic layers over anhydrous sodium sulfate. Evaporate the dichloromethane under vacuum at 32-35°, take up the residue in mobile phase, clean up further using a Sep-Pak SPE cartridge, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 60:40
Flow rate: 1
Injection volume: 5
Detector: UV 224

CHROMATOGRAM

Limit of detection: 20 ppb

KEY WORDS

SPE

REFERENCE

Thapar,S.; Bhushan,R.; Mathur,R.P. Degradation of organophosphorus and carbamate pesticides in soils -HPLC determination, *Biomed.Chromatogr.*, **1995**, *9*, 18–22.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Spherisorb octyl
Mobile phase: MeCN:water 42:58 containing 100 mM n-butylamine, pH adjusted to 3.0 with perchloric acid
Flow rate: 1
Detector: UV 313

CHROMATOGRAM

Retention time: 11.40

OTHER SUBSTANCES

Simultaneous: oxantel, pyrantel

KEY WORDS

protect from light

REFERENCE

Allender,W.J. High-performance liquid chromatographic determination of oxantel and pyrantel pamoate, *J.Chromatogr.Sci.*, **1988**, *26*, 470–472.

SAMPLE

Matrix: solutions

Sample preparation: Pass 100 mL water through column A at 5 mL/min then elute the contents of column A onto column B with the mobile phase, elute column B with the mobile phase and monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 4.6 5 μm Spherisorb ODS C18; B 250 × 4.6 5 μm Supelcosil LC-8 C8
Mobile phase: Gradient. MeCN:water 30:70 for 5 min, to 60:40 over 10 min, maintain at 60:40 for 10 min, to 30:70 over 5 min, maintain at 30:70 for 5 min and inject next sample.
Flow rate: 1.5
Injection volume: 100000
Detector: UV 220

CHROMATOGRAM
Retention time: 17.35
Limit of detection: 10 pg/mL

OTHER SUBSTANCES
Simultaneous: propoxur, carbofuran, captan, propham, chloropropham, barban, butylate

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KEY WORDS
water; drinking water; column-switching

REFERENCE
Marvin,C.H.; Brindle,I.D.; Hall,C.D.; Chiba,M. Development of an automated high-performance liquid chromatographic method for the on-line pre-concentration and determination of trace concentrations of pesticides in drinking water, *J.Chromatogr.*, **1990**, *503*, 167–176.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 1 mg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 2 octadecylsilyl (Hewlett-Packard 79916 OD-552)
Mobile phase: MeOH:water 60:40
Column temperature: 25
Flow rate: 1.5
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: atrazine, DPX-4189, folpet, linuron

REFERENCE
Wang,Q-S.; Gao,R.-Y.; Wang,H.-Y. Computer-assisted optimization of selectivity (mobile phase, pH, and ion concentration) in high-performance liquid chromatography, *J.High Res.Chromatogr.*, **1990**, *13*, 173–177.

SAMPLE
Matrix: solutions
Sample preparation: Equilibrate column A with 10 mL MeCN and 10 mL water (pH 7). Pump 200 mL drinking water through column A at 3 mL/min, back flush contents of column A onto column B with the mobile phase and start the gradient.

HPLC VARIABLES

Column: A 10 × 2.1 5 μm RP-18 octadecylsilica (E. Merck); B 150 × 4.6 5 μm Nucleosil C18
Mobile phase: Gradient. MeCN:water from 40:60 to 60:40 over 15 min
Injection volume: 200000
Detector: UV 254

CHROMATOGRAM

Retention time: 8.7
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES

Extracted: azinphos-methyl, phosmet, parathion-methyl, azinphos-ethyl, fenitrothion, parathion, diazinon

KEY WORDS

drinking water; column-switching

REFERENCE

Driss,M.R.; Hennion,M.-C.; Bouguerra,M.L. Determination of carbaryl and some organophosphorus pesticides in drinking water using on-line liquid chromatographic preconcentration techniques, *J.Chromatogr.*, **1993**, *639*, 352–358.

SAMPLE

Matrix: solutions
Sample preparation: Condition a 10 × 4 55 mg 40 μm C18/OH Bondesil SPE cartridge (Varian/Analytichem) with 1 mL MeOH and 1 mL water, pass through 5 mL test water at 1 mL/min, pass through 500 μL pure water, elute the contents of the SPE cartridge onto the analytical column with mobile phase.

HPLC VARIABLES

Guard column: 10 × 4 4 μm Supersphere RP-8 (Merck)
Column: 250 × 4 4 μm Supersphere RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:water 20:80 containing 2.5 mM sodium acetate. B was MeOH:water 20:80 containing 2.5 mM sodium acetate. C was MeCN:water 60:40 containing 2.5 mM sodium acetate. A:B:C 75:25:0 for 5 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 5 min, re-equilibrate at initial conditions for 15 min.
Column temperature: 35
Flow rate: 0.75
Injection volume: 100
Detector: F ex 340 em 445 following post-column reaction. The column effluent flowed through a 50 × 4 Aminex A-27 (Bio-Rad) column at 120-140° and was mixed with reagent pumped at 1 mL/min, this mixture flowed through a 200 × 0.12 PTFE tube to the detector. (Reagent was prepared by adding 2 mL 25 mg/mL o-phthalaldehyde in MeCN and 100 μL 2-mercaptoethanol to 200 mL 5 mg/mL disodium tetraborate in water then making up to 250 mL with water.)

CHROMATOGRAM

Retention time: 24.53
Internal standard: trimethacarb (26.12)
Limit of detection: 0.03-0.05 ng/mL

OTHER SUBSTANCES

Simultaneous: aldicarb, bendiocarb, bufencarb, butocarboxim, carbanolate, carbofuran, cloethocarb, dioxacarb, ethiofencarb, fenobucarb, isoprocarb, methiocarb, methomyl, oxamyl, promecarb, propoxur, thiofanox, tranid

KEY WORDS
water; SPE; post-column reaction

REFERENCE
Hiemstra,M.; de Kok,A. Determination of N-methylcarbamate pesticides in environmental water samples using automated on-line trace enrichment with exchangeable cartridges and high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *667*, 155–166.

SAMPLE
Matrix: solutions
Sample preparation: Flush column A with 5 mL MeOH and 5 mL MeOH:pH 5.0 ammonium acetate, pass a 100 mL sample through the column at 4 mL/min, backflush the contents of column A onto column B and start the gradient, monitor the effluent from column B.

HPLC VARIABLES
Column: A 10 × 2 15-25 μm PLRP-S styrene-divinylbenzene co-polymer (Spark Holland); B 200 × 4 5 μm Spherisorb ODS2
Mobile phase: MeOH:100 mM pH 5.0 ammonium acetate from 30:70 to 88:12 over 34 min.
Column temperature: 40
Flow rate: 0.4
Injection volume: 100000
Detector: UV 280 or MS, Hewlett-Packard 5989 A, dual EI/chemical ionization source, ion source block 250°, quadrupole 100°, m/z 64-400, desolvation chamber 65°, helium nebulizer 50 psi, second-stage momentum separator 0.5 Torr, ion source chamber 15 μTorr

CHROMATOGRAM
Retention time: 22
Limit of detection: <1 ng/mL

OTHER SUBSTANCES
Simultaneous: propoxur, aldicarb, atrazine, barban, carbofuran, cyanazine, diuron, fluometuron, linuron, methomyl, monuron, oxamyl, simazine

KEY WORDS
water; column-switching

REFERENCE
Marcé,R.M.; Prosen,H.; Crespo,C.; Calull,M.; Borrull,F.; Brinkman,U.A.T. On-line trace enrichment of polar pesticides in environmental waters by reversed-phase liquid chromatography-diode array detection-particle beam mass spectrometry, *J.Chromatogr.A*, **1995**, *696*, 63–74.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 3 5 μm Kromasil-100-C18 (Akzo Nobel)
Column: 150 × 4 5 μm Kromasil-100-C18 (Akzo Nobel)
Mobile phase: MeCN:buffer 28:72 (Buffer was 820 mg/L sodium tetraborate decahydrate containing 50 μg/mL phthalaldehyde and 0.06 μL/mL 2-mercaptoethanol, adjusted to pH 8.5 with 100 mM HCl.)
Flow rate: 1
Injection volume: 20
Detector: F ex 340 em 460 following post-column reaction. The column effluent flowed through a 3 m × 0.51 mm ID stainless steel tube at 140° to the detector. (Although the reagents are in the mobile phase the derivatization reaction does not take place until the

post-column reactor where the insecticides are hydrolyzed to methylamine that is then derivatized. This procedure avoids the use of a second pump for the post-column reagent.)

CHROMATOGRAM

Retention time: 19
Limit of detection: 700 pg

OTHER SUBSTANCES

Simultaneous: aldicarb, butocarboxim, carbofuran, dioxacarb, methomyl, propoxur

KEY WORDS

post-column reaction

REFERENCE

Sabala,A.; Portillo,J.L.; Broto-Puig,F.; Comellas,L. Development of a new high-performance liquid chromatography method to analyse N-methylcarbamate insecticides by a simple post-column derivatization system and fluorescence detection, *J.Chromatogr.A*, **1997**, *778*, 103–110.

SAMPLE

Matrix: tissue
Sample preparation: 21 g Liver + 60 g anhydrous sodium sulfate, mix with spatula, add 200 mL dichloromethane, mix with spatula, homogenize (VirTis 45) for 2 min at medium speed, filter through 5 g anhydrous sodium sulfate, re-extract tissue and sodium sulfate with 100 mL dichloromethane, filter, wash out flask with 25 mL dichloromethane, filter. Combine filtrates and filter them through 2 g anhydrous sodium sulfate, rinse flask with 20 mL dichloromethane, wash filter with 10 mL dichloromethane. Concentrate filtrate to 1-2 mL under reduced pressure at 30° (do not allow to go dry), transfer residue to a tube with 1-2 mL cyclohexane, wash in with dichloromethane:cyclohexane 50:50, make volume in tube 7.5 mL, filter (0.45 μm), add 5 mL to a 600 × 25 tube containing 60 g 200-400 mesh BioBeads SX-3 resin (Analytical BioChemistry Laboratories), pump through at 5 mL/min with dichloromethane:cyclohexane 50:50 mobile phase, collect fraction containing the compound, evaporate under reduced pressure at 30° to about 1 mL, make up to 2 mL with dichloromethane, add 1 mL to 1 mL 100 mg Bond Elut aminopropyl SPE cartridge (previously conditioned with 1 mL dichloromethane), elute with 3-5 mL dichloromethane: MeOH 98.5:1.5, evaporate eluate to dryness at 30° under reduced pressure (do not over dry), reconstitute in 200 μL MeOH, vortex for 5 s, filter (0.45 μm), inject a 20-30 μL aliquot.

HPLC VARIABLES

Guard column: Guard-PAK (Waters no. 88070)
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: Gradient. MeCN:water from 12:88 to 70:30 over 30 min, to 80:20 over 1 min, maintain at 80:20 for 8 min, re-equilibrate at initial conditions for 10 min.
Flow rate: 1.5
Injection volume: 20-30
Detector: F ex 340 em 418, following post-column derivatization. The column effluent is mixed with 50 mM NaOH at 0.27 mL/min and the mixture flows through a 1 mL coil at 80° and is mixed with 140 μg/mL o-phthalaldehyde and 1 mL/L mercaptoethanol in 50 mM pH 10.5 potassium borate buffer pumped at 0.27 mL/min. The mixture flows through a 1 mL coil at 40° to the detector.

CHROMATOGRAM

Retention time: 23.1
Limit of quantitation: 5 ppb

OTHER SUBSTANCES

Extracted: methomyl, aldicarb, carbofuran, methiocarb, bufencarb

KEY WORDS
liver; pig; cow; duck; SPE; post-column reaction; derivatization

REFERENCE
Ali,M.S. Determination of *N*-methylcarbamate pesticides in liver by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1989**, *72*, 586–592.

SAMPLE
Matrix: water
Sample preparation: Extract 500 mL water with two 25 mL portions of dichloromethane, combine the extracts and dry them over anhydrous sodium sulfate for 10 min, evaporate to dryness under a stream of air, reconstitute with 40 μL acetone, add 300 μL 100 mM sodium carbonate, heat at 45-50° for 30-40 min, cool, add 300 μL acetone, add 100 μL 0.2% dansyl chloride in acetone, mix well, heat at 45° for 20 min, cool, evaporate the acetone under a stream of air, extract with 300 μL benzene (Caution! Benzene is a carcinogen!). Remove the organic layer and dry it over anhydrous sodium sulfate, inject a 1-10 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.4 Zipax coated with 0.5% β,β'-oxydipropionitrile
Mobile phase: Hexane:EtOH 95:5
Injection volume: 1-10
Detector: F primary filter Turner 810, secondary filter Turner 827

CHROMATOGRAM
Retention time: k' 0.59

OTHER SUBSTANCES
Simultaneous: aldicarb (Temik), carbofuran, Carzol, dimethylamine, methomyl, methylamine, Mobam, propoxur (Baygon)

KEY WORDS
lake water; derivatization; normal phase

REFERENCE
Frei,R.W.; Lawrence,J.F.; Hope,J.; Cassidy,R.M. Analysis of carbamate insecticides by fluorigenic labelling and high-speed liquid chromatography, *J.Chromatogr.Sci.*, **1974**, *12*, 40–44.

SAMPLE
Matrix: water
Sample preparation: Condition a 4.6 mm dia C18 Empore SPE extraction disk with 10 mL MeOH at 1 mL/min and 10 mL water at 1 mL/min. Pass 10 mL water through the disk, backflush the contents of the disk on to the column with mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 4 μm Supersphere 60 RP-8 (Merck)
Mobile phase: Gradient. A was MeCN:MeOH:water 40:40:20. B was MeCN:water 10:90. A:B from 5:95 to 20:80 over 15 min, to 30:70 over 20 min, to 65:35 over 20 min, to 100:0 over 7 min, return to initial conditions over 5 min, re-equilibrate for 10 min.
Flow rate: 0.8
Detector: F ex 330 em 465 following post-column reaction (LC.GC 1988, 6, 994) using thiofluor instead of 2-mercaptoethanol

CHROMATOGRAM
Retention time: 8 (aldicarb sulfoxide), 12 (aldicarb sulfone), 22.5 (3-hydroxycarbofuran), 37 (aldicarb), 40 (3-ketocarbofuran), 50 (carbofuran), 52.5 (carbaryl), 54 (1-naphthol)
Limit of detection: 5-40 pg/mL

OTHER SUBSTANCES
Extracted: aldicarb sulfoxide, aldicarb sulfone, aldicarb sulfoxide, carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, 1-naphthol

KEY WORDS
derivatization; SPE; post-column reaction

REFERENCE
Chiron,S.; Barceló,D. Determination of pesticides in drinking water by on-line solid-phase disk extraction followed by various liquid chromatographic systems, *J.Chromatogr.*, **1993**, *645*, 125–134.

SAMPLE
Matrix: water
Sample preparation: Filter, inject a 400 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: C18
Column: 150 × 4.6 3 μm HS-3C18 (Perkin Elmer)
Mobile phase: Gradient. MeCN:water from 5:95 to 20:80 over 13 min, to 65:35 over 15 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 8 min.
Flow rate: 1
Injection volume: 400
Detector: F ex 340 em 460 following post-column reaction. The column effluent mixed with the reagent pumped at 0.1 mL/min and the mixture flowed through a 500 μL reaction coil at 95° to the detector. (Prepare the reagent by adding 1.25 mL 10 M NaOH to 100 mL water, add 10 mL 18 mg/mL N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor; Pickering Laboratories, Mountain Vie CA), add 2.5 mL 10 mg/mL o-phthalaldehyde in MeOH, make up to 250 mL with water, filter (0.45 μm nylon), degas with helium for 10 min before use. Prepare fresh each day.)

CHROMATOGRAM
Retention time: 29.78
Internal standard: 4-bromo-3,5-dimethylphenyl N-methylcarbamate (34)
Limit of detection: 0.4 ng/mL

OTHER SUBSTANCES
Simultaneous: aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, 3-hydroxycarbofuran, methiocarb, methomyl, oxamyl, propoxur

KEY WORDS
post-column reaction

REFERENCE
Simon,V.A.; Pearson,K.S.; Taylor,A. Determination of N-methylcarbamates and N-methylcarbamoyloximes in water by high performance liquid chromatography with the use of fluorescence detection and a single o-phthalaldehyde post-column reaction, *J.Chromatogr.*, **1993**, *643*, 317–320.

SAMPLE
Matrix: water
Sample preparation: Prepare an SPE column by placing 500 mg 37-55 μm Bondapak octadecylsilica bonded-phase material in a 100 × 10 glass column fitted with a 40-100 μm glass fritted disc. Condition the SPE column with 5 mL MeOH and 10 mL water, do not allow to go dry. Add NaCl to the water sample so as to achieve a concentration of 10%, pass a 250 mL aliquot through the SPE column, elute with 10 mL MeCN:dichloromethane 50:50, evaporate the eluate at 60°, make up to 200 μL with MeCN, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 reversed-phase (Phase Sep)
Column: 250 × 4 Spherisorb C18
Mobile phase: Gradient. MeCN:water 23:77 for 0.1 min, to 45:55 over 14.9 min, to 70:30 over 25 min, re-equilibrate at initial conditions for 10 min.
Injection volume: 20
Detector: UV 210

CHROMATOGRAM

Retention time: 20
Limit of detection: 0.09 ng/mL

OTHER SUBSTANCES

Simultaneous: carbendazim, carbofuran, dietofencarb, dioxacarb, fenothiocarb, iprodione, methomyl, methylthiofanate, molinate, oxamyl, thiobencarb

KEY WORDS

SPE; surface water

REFERENCE

Jiménez,B.; Moltó,J.C.; Font,G. Influence of dissolved humic material and ionic strength on C8 extraction of pesticides from water, *Chromatographia*, **1995**, *41*, 318–324.

SAMPLE

Matrix: water
Sample preparation: Mix water sample with 2 mL 500 mM NaOH, make up to 40 mL with water, shake for a few s, let stand for 10 min, add 1 mL glacial acetic acid, make up to 50 mL with water, filter (0.45 μm), sonicate the filtrate, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 33 × 4.6 3 μm Pecosphere 3x3 CR C18
Mobile phase: MeCN:water:glacial acetic acid 50:49.5:0.5 containing 10 mM sodium perchlorate
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, model 5021 conditioning cell 0 V, model 5011 dual analytical cell with porous graphite working electrodes at +0.1 V and +0.6 V (monitored), cells protected with 0.2 μm porous graphite filters

CHROMATOGRAM

Retention time: 1.21
Limit of detection: 0.98 nM

OTHER SUBSTANCES

Simultaneous: carbofuran, fenobucarb

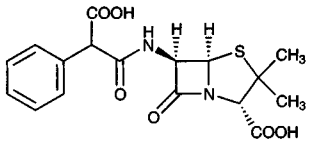
KEY WORDS

derivatization; river water

REFERENCE

Galeano Díaz,T.; Guiberteau,A.; Salinas,F.; Ortiz,J.M. Rapid and sensitive determination of carbaryl, carbofuran and fenobucarb by liquid chromatography with electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2681–2690.

Carbenicillin



Molecular formula: $C_{17}H_{18}N_2O_6S$
Molecular weight: 378.41
CAS Registry No.: 4697-36-3, 4800-94-6 (disodium salt), 17230-86-3 (potassium salt)
Merck Index: 1838
Lednicer No.: 1 414

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. 200 μL Serum + 200 μL 10 M urea, mix, filter (Amicon MPS-1 micropartition system with Amicon YMT membranes) while centrifuging at 1500 g for 10 min. Add 200 μL of the ultrafiltrate to 200 μL reagent and heat at 60° for 10 min, cool to room temperature, inject a 30-90 μL aliquot. Urine. Dilute urine 10-fold with water, filter (0.45 μm acrylate copolymer). Add 200 μL of the filtrate to 200 μL reagent and heat at 60° for 10 min, cool to room temperature, inject a 30-60 μL aliquot. (Prepare reagent by dissolving 13.81 g 1,2,4-triazole in 60 mL water, add 10 mL 2.7 mg/mL mercury(II) chloride in water, adjust pH to 9.0 ± 0.05 with saturated NaOH, make up to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Develosil ODS-5 (Nomura Chemicals)
Mobile phase: MeCN:buffer containing 5 mM tetrabutylammonium bromide and 5 mM sodium thiosulfate 1:1.8 (Prepare the buffer by dissolving 14.32 g $Na_2HPO_4.12H_2O$ and 6.240 g $NaH_2PO_4.2H_2O$ in 1 L water then diluting 100-fold.)
Column temperature: 40
Flow rate: 1
Injection volume: 30-90
Detector: UV 328

CHROMATOGRAM

Retention time: 4.5
Limit of detection: 1 μg/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES

Interfering: ticarcillin

KEY WORDS

serum; derivatization; ultrafiltrate

REFERENCE

Haginaka,J.; Wakai,J. High-performance liquid chromatographic assay of carbenicillin, ticarcillin and sulbenicillin in serum and urine using pre-column reaction with 1,2,4-triazole and mercury(II) chloride, *Analyst*, **1985**, *110*, 1185–1188.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a Bond Elut SAX SPE cartridge with 2 mL MeOH and 2 mL water. Plasma. Filter plasma (0.45 μm, Cosmonice, type W, Millipore), add 500 (human), 300 (rabbit) or 100 (rat) μL filtrate to ten volumes 50 mM ammonium acetate, add the mixture to the SPE cartridge, wash with 3 mL MeCN:500 mM acetic acid 50:50, wash with 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 500 μL MeOH:10% LiCl 40:60, inject a 20 μL aliquot. Urine. Dilute urine 40-fold with water, filter (0.45 μm, Cosmonice, type W, Millipore), add 500 μL filtrate to 5 mL 50 mM ammonium acetate, add the mixture to the SPE cartridge, wash with 3 mL MeCN:500 mM acetic acid 50:50,

wash with 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 1 mL MeOH:10% LiCl 40:60, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil 5C18-AR (Nacalia Tesque)
Mobile phase: MeOH:50 mM ammonium acetate 1:9 (human), 1:6 (rabbit), 1:7 (rat)
Flow rate: 1.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 18 (R), 24 (S)
Limit of detection: 10000 ng/mL

KEY WORDS
plasma; chiral; rat; rabbit; human; SPE

REFERENCE
Ishida,M.; Tsuda,Y.; Onuki,Y.; Itoh,T.; Shimada,H.; Yamada,H. Determination of carbenicillin epimers in plasma and urine with high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *652*, 43–49.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: 10 mM ammonium acetate
Flow rate: 1.6
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 5, 6 (two isomers)

OTHER SUBSTANCES
Interfering: ticarcillin

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE
Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil C18-AR (Nacalai Tesque Co., Kyoto)
Mobile phase: MeOH:50 mM pH 7.0 10:80 or MeOH:50 mM ammonium acetate 10:70
Flow rate: 0.9
Injection volume: 20-40
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: sulbenicillin

KEY WORDS
carbenicillin is IS

REFERENCE
Itoh,T.; Watanabe,N.; Ishida,M.; Tsuda,Y.; Koyano,S.; Tsunoi,T.; Shimada,H.; Yamada,H. Stereoselective disposition of sulbenicillin in humans, *Antimicrob.Agents Chemother.*, **1998**, *42*, 325–331.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: dicloxacillin, methicillin, penicillin G, penicillin V, cloxacillin, nafcillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10

Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 13.7

OTHER SUBSTANCES

Simultaneous: cephapirin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

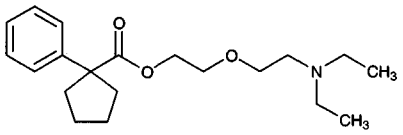
KEY WORDS

derivatization

REFERENCE

Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

Carbetapentane



Molecular formula: $C_{20}H_{31}NO_3$
Molecular weight: 333.47
CAS Registry No.: 77-23-6, 23142-01-0 (citrate)
Merck Index: 1840

SAMPLE

Matrix: formulations
Sample preparation: Mix 2-4 mL syrup or expectorant oral solution with 4 mL 10 μg/mL IS in MeOH. Make up to 25 mL with MeOH. Inject a 20 μL. aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm LiChrosphere 100RP-18
Mobile phase: MeOH:25% (w/w) ammonia 99.2:0.8
Flow rate: 1.2
Injection volume: 20
Detector: UV 258

CHROMATOGRAM

Retention time: k' 1.95
Internal standard: chlorpromazine (k' 2.78)
Limit of detection: 8 μg/mL

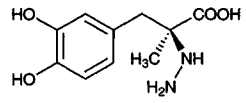
KEY WORDS

syrup; oral solution

REFERENCE

Gad-Kariem,E.A.; Abounassif,M.A. Determination of pentoxyverine in cough preparations by high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3049–3059.

Carbidopa



Molecular formula: $C_{10}H_{14}N_2O_4$
Molecular weight: 226.23
CAS Registry No.: 28860-95-9, 38821-49-7 (monohydrate)
Merck Index: 1843
Lednicer No.: 2 119

SAMPLE

Matrix: blood
Sample preparation: 0.5 mL Plasma + 50 μL 4 M perchloric acid + 50 μL 1 μg/mL dihydroxybenzylamine in 0.1 M perchloric acid, centrifuge at 1500 g for 10 min. Remove 300 μL supernatant and centrifuge it at 1600 g through a 0.2 μm regenerated cellulose filter, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Biophase ODS + 50 × 4.6 Pelliguard LC-18
Column: 250 × 4.6 5 μm Biophase ODS or 250 × 4.6 Phase II ODS (both from Bioanalytical Systems)
Mobile phase: MeOH:buffer 5:95 (Buffer was 20 mM sodium citrate, 100 mM NaH_2PO_4, 0.15 mM, and 1.25 mM heptanesulfonic acid, pH 3.2.)
Column temperature: 28
Flow rate: 1-1.5
Injection volume: 20
Detector: E, Bioanalytical Systems LC-150 in dual-parallel mode, channel 1 700 mV 200 nA f.s. for levodopa and 3-O-methyldopa, channel 2 560 mV 10 nA f.s. for dopamine, carbidopa, and dihydroxyphenylacetic acid, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 15.1
Internal standard: dihydroxybenzylamine (7)
Limit of detection: 6 ng/mL

OTHER SUBSTANCES

Simultaneous: 3-O-methyldopa, dopamine, levodopa, dihydroxyphenylacetic acid

KEY WORDS

plasma

REFERENCE

Cedarbaum,J.M.; Williamson,R.; Kutt,H. Simultaneous determination of levodopa, its metabolites and carbidopa in clinical samples, *J.Chromatogr.*, **1987**, *415*, 393–399.

SAMPLE

Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex pre-swollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. Add 1 mL plasma to column, elute with 5.5 mL water, discard first 1 mL. Add next 4.5 mL to 0.5 mL 0.5 M perchloric acid, centrifuge, inject 10 μL aliquot of supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid

Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 13
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: O-methyldopa, levodopa, dihydroxyphenylacetic acid (DOPAC)

KEY WORDS
plasma

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 100 μL 4 M perchloric acid, vortex, centrifuge at 2000 g for 10 min, inject a 60 μL aliquot of the supernatant (keep sample tray at 6 ± 1°).

HPLC VARIABLES
Guard column: 45 × 4.6 37-40 μm Whatman pellicular-ODS followed by 45 × 4.6 5 μm Ultrasphere-IP C18
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeOH:20 mM orthophosphoric acid and 4 mM sodium octanesulfonate 25:75 adjusted to pH 2.8 ± 0.05 with 50% NaOH
Column temperature: 40
Flow rate: 1
Injection volume: 60
Detector: E, BAS LC-4B, 0.75 V vs Ag/AgCl, 5 nA full scale for carbidopa, 20 nA full scale for 3-O-methyldopa and levodopa

CHROMATOGRAM
Retention time: 10.9
Internal standard: α-ethyldopa (15.4)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES
Extracted: levodopa, 3-O-methyldopa

KEY WORDS
plasma; stabilize plasma sample immediately with EDTA and 2 mg/mL sodium metabisulfite

REFERENCE
Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

SAMPLE
Matrix: blood

Sample preparation: 4 mL Plasma + 500 μL 20 mg/mL ascorbic acid solution, vortex for 30 s. 1 mL Aliquot + 75 mg acid washed alumina + 100 μL 1 μg/mL 3,4-dihydroxybenzylamine hydrobromide in buffer, vortex, add 1 mL 1.5 M pH 8.6 TRIS buffer, shake at 230 oscillations/min for 15 min. Allow to settle and discard plasma, wash the alumina twice by shaking with 5 mL water for 10 min. To the washed alumina add 900 μL buffer, vortex for 20 s, allow to settle, inject a 50 μL aliquot of the supernatant. (Buffer was 200 mM phosphoric acid containing 3.3 μm EDTA and 6.7 μm potassium metabisulfite.)

HPLC VARIABLES

Column: 100 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:buffer 8:92 (Buffer was pH 2.6 550 mM NaH_2PO_4 containing 1 mM sodium octyl sulfate and 0.7 mM EDTA.)
Flow rate: 1.5
Injection volume: 50
Detector: E, Bioanalytical Systems LC-4B, glassy carbon electrode, Ag/AgCl reference electrode, 0.75 V.

CHROMATOGRAM

Retention time: 9.7
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (4.5)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: levodopa
Noninterfering: caffeine, ibuprofen, aspirin, nicotine, acetaminophen, theophylline

KEY WORDS

plasma; SPE

REFERENCE

Miller,R.B.; Dehelean,L.; Bélanger,L. Determination of carbidopa and levodopa in human plasma by high-performance liquid chromatography with electrochemical detection, *Chromatographia*, **1993**, *35*, 607–612.

SAMPLE

Matrix: formulations
Sample preparation: Powder levodopa/carbidopa tablets or contents of capsules, weigh out an amount equivalent to about 100 mg levodopa, add 30 mL 0.1 M HCl, sonicate, make up to 50 mL with 0.1 M HCl, mix, filter (0.45 μm), discard first 5 mL filtrate. 10 mL Filtrate + 50 mL 0.5 mg/mL acetaminophen in MeOH:mobile phase 75:175, make up to 100 mL with mobile phase, mix, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: 3% aqueous acetic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM

Retention time: 5
Internal standard: acetaminophen (9)

OTHER SUBSTANCES

Simultaneous: levodopa

KEY WORDS

capsules; tablets

REFERENCE
Ting,S. Liquid chromatographic determination of levodopa and levodopa-carbidopa in solid dosage forms: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 987–990.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve in mobile phase, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeOH:50 mM ammonium acetate adjusted to pH 4.1 with 0.6 M acetic acid 1:99
Flow rate: 0.9
Detector: E, Coulochem model 5100A, screen electrode +0.3 V, sample electrode +0.6 V and UV 280

CHROMATOGRAM
Retention time: 11.4
Limit of detection: 200 ng/mL (UV), 2 ng/mL (E)

OTHER SUBSTANCES
Simultaneous: hydroxydopa, levodopa, methyldopa, methoxytyrosine, methylcarbidopa, impurities

KEY WORDS
stability-indicating; tablets

REFERENCE
Kafil,J.B.; Dhingra,B.S. Stability-indicating method for the determination of levodopa, levodopa-carbidopa and related impurities, *J.Chromatogr.A*, **1994**, *667*, 175–181.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 4.8
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Simultaneous: dopamine, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, norepinephrine, phenylephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE

Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE

Matrix: urine

Sample preparation: 100 μL Urine + 100 μL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA + 25 μL 1.25 μg/mL α-ethyldopa in 0.1 M HCl + 25 mg alumina + 1 mL 2 M pH 8.6 Tris-HCl buffer in a microfilter tube (Centrex, Schleicher & Schuell), vortex 5 min, allow to stand for 10 min, filter off water, wash with 5 mL water, add 5 mL water, centrifuge at 3000 g, vortex with 400 μL 0.2 M perchloric acid containing 11 mM disodium EDTA and 0.4 M sodium metabisulfite, centrifuge at 9000 g for 5 min, inject 50 μL of filtrate.

HPLC VARIABLES

Guard column: 40 × 4.6 Bio-Sil ODS-10 (Bio-Rad)

Column: 250 × 4.6 5 μm Ultrasphere IP C18

Mobile phase: MeOH:water 22.5:77.5 containing 20 mM citric acid, 20 mM Na_2HPO_4, 4 mM sodium octanesulfonate, and 0.05 mM disodium EDTA, pH adjusted to 2.74 ± 0.01 with 2 M citric acid

Column temperature: 40

Injection volume: 50

Detector: E, BAS LC-4B, 0.54 V vs Ag/AgCl, 50 nA full scale

CHROMATOGRAM

Retention time: 10

Internal standard: α-ethyldopa (14)

Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Extracted: levodopa, dopamine

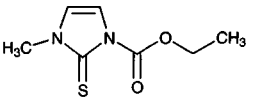
KEY WORDS

stabilize each 10 mL urine sample immediately with 0.5 mL 0.1 M HCl and 1 mL solution containing 55 mM ascorbic acid and 55 mM disodium EDTA; SPE

REFERENCE

Titus,D.C.; August,T.F.; Yeh,K.C.; Eisenhandler,R.; Bayne,W.F.; Musson,D.G. Simultaneous high-performance liquid chromatographic analysis of carbidopa, levodopa and 3-O-methyldopa in plasma and carbidopa, levodopa and dopamine in urine using electrochemical detection, *J.Chromatogr.*, **1990**, *534*, 87–100.

Carbimazole



Molecular formula: $C_7H_{10}N_2O_2S$
Molecular weight: 186.23
CAS Registry No.: 22232-54-8
Merck Index: 1844
Lednicer No.: 1 240

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 11.138

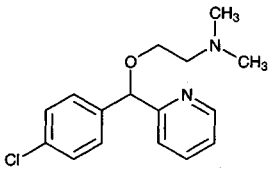
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Carbinoxamine



Molecular formula: $C_{16}H_{19}ClN_2O$
Molecular weight: 290.79
CAS Registry No.: 486-16-8, 3505-38-2 (maleate), 49746-00-1 (l-form tartrate)
Merck Index: 1845
Lednicer No.: 1 43

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 30 μL 1 μg/mL phenyltoloxamine in water + 200 μL ammonia, extract twice with 7 mL pentane:diethyl ether 75:25. Combine the organic layers and evaporate them to dryness, reconstitute the residue in 150 μL mobile phase, inject a 90 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Spherisorb CN cyanopropyl
Mobile phase: MeCN:5 mM pH 6 phosphate buffer 40:60
Flow rate: 1
Injection volume: 90
Detector: E, Environmental Science Associates Coulochem model 5010, screen mode +0.55 V and +0.90 V

CHROMATOGRAM

Retention time: 15
Internal standard: phenyltoloxamine (19)
Limit of detection: 0.5 ng/mL
Limit of quantitation: 2 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Stockis,A.; Deroubaix,X.; Jeanbaptiste,B.; Lins,R.; Allemon,A.M.; Laufen,H. Relative bioavailability of carbinoxamine and phenylephrine from a retard capsule after single and repeated dose administration in healthy subjects, *Arzneimittelforschung*, **1995**, *45*, 1009–1012.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 226

CHROMATOGRAM

Retention time: 5.44
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using

a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 12.81

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 1.98

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 cellulose tris(3,5-dimethylphenylcarbamate)
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 0.5
Detector: UV

CHROMATOGRAM
Retention time: k' 0.98 (of first (+) enantiomer)

KEY WORDS
chiral; α 1.31

REFERENCE
Okamoto,Y.; Aburatani,R.; Hatano,K.; Hatada,K. Optical resolution of racemic drugs by chiral HPLC on cellulose and amylose tris(phenylcarbamate) derivatives, *J.Liq.Chromatogr.*, **1988**, *11*, 2147–2163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chiracel OD
Mobile phase: Hexane:isopropanol 90:10
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 5.25, 6.25 (enantiomers)

KEY WORDS
chiral

REFERENCE
Baxter Scientific Products Catalog, 1992-3, p. 213, p. 213.

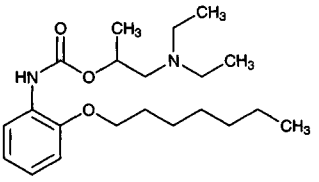
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Chirex 3014 (Phenomenex)
Mobile phase: Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 40:50:10 (EtOH/trifluoroacetic acid was premixed 20:1.)
Flow rate: 0.7-1
Injection volume: 20
Detector: UV 262

KEY WORDS
chiral; α = 1.15 for enantiomers

REFERENCE
Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

Carbisocaine



Molecular formula: $C_{21}H_{36}N_2O_3$
Molecular weight: 364.52
CAS Registry No.: 76629-87-3, 68931-03-3 (HCl)

SAMPLE
Matrix: bulk

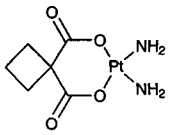
HPLC VARIABLES
Column: 250 × 4 5 μm LiChroCART ChiraDex (Merck)
Mobile phase: MeCN:0.03% pH 6.1 triethylamine 5:95
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 26 (R-(-)), 28 (S-(+))

KEY WORDS
chiral

REFERENCE
Cizmárik,J.; Lehotay,J.; Hromul'áková,K.; Pokorná,M.; Lacuska,M. HPLC separation of enantiomers of carbisocaine. Study of local anaesthetics, part 138, *Pharmazie*, **1997**, *52*, 402–403.

Carboplatin



Molecular formula: $C_6H_{12}N_2O_4Pt$
Molecular weight: 371.25
CAS Registry No.: 41575-94-4
Merck Index: 1870
Lednicer No.: 4 16

SAMPLE

Matrix: blood
Sample preparation: Inject an aliquot of plasma ultrafiltrate directly (Further sample clean up not required when using post-column reaction detection.).

HPLC VARIABLES

Column: 150 × 4.6 3 μm YMC ODS-AQ
Mobile phase: 20 mM NaH_2PO_4
Flow rate: 0.7
Detector: UV 290 following post-column reaction detection. The column effluent mixed with 20 mM pH 5.4 NaH_2PO_4 containing 40 mM sodium bisulfite pumped at 0.7 mL/min and the mixture flowed through a 15.2 m × 0.5 mm ID knitted PTFE coil to the detector.

CHROMATOGRAM

Limit of detection: 13 ng/mL
Limit of quantitation: 50 ng/mL

KEY WORDS

plasma; ultrafiltrate; post-column reaction

REFERENCE

Burns,R.B.; Embree,L. Comparison of ultraviolet (UV) and post-column reaction (PC/UV) detection methods for the high-performance liquid chromatographic (HPLC) analysis of carboplatin in human plasma ultrafiltrate (PUF) (Abstract 2483), *Pharm.Res.*, **1997**, *14*, S373–S373.

SAMPLE

Matrix: formulations
Sample preparation: Dilute carboplatin injections with water, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak ODS
Mobile phase: Gradient. A was MeOH. B was 0.02% aqueous formic acid. A:B 0:100 for 6 min, from 0:100 to 25:75 in 1 min, maintain at 25:75 for 8 min
Flow rate: 0.5
Injection volume: 20
Detector: MS, Fisons VG Quattro Quadropole, electrospray, source 100°, cone voltage 30 V, scan m/z 200-1000, m/z 372

CHROMATOGRAM

Retention time: 5.3
Limit of detection: 35 ng/mL

OTHER SUBSTANCES

Simultaneous: DWA2114R

KEY WORDS

injections

REFERENCE
Burns,R.B.; Burton,R.W.; Albon,S.P.; Embree,L. Liquid chromatography-mass spectrometry for the detection of platinum antineoplastic complexes, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 367–372.

SAMPLE
Matrix: formulations
Sample preparation: Dilute carboplatin injections with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak ODS
Mobile phase: MeCN:0.02% aqueous formic acid 2:98
Flow rate: 0.5
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 5.4
Limit of detection: 200 ng/mL

KEY WORDS
injections

REFERENCE
Burns,R.B.; Burton,R.W.; Albon,S.P.; Embree,L. Liquid chromatography-mass spectrometry for the detection of platinum antineoplastic complexes, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 367–372.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4.6 5 μm C18
Mobile phase: water
Flow rate: 2.5
Injection volume: 20
Detector: UV 228

CHROMATOGRAM
Retention time: 3.10

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 3 μm YMC ODS-AQ
Mobile phase: MeCN:20 mM NaH_2PO_4 1.3:98.7
Flow rate: 0.7
Detector: UV 230

CHROMATOGRAM

Limit of detection: 25 ng/mL
Limit of quantitation: 75 ng/mL

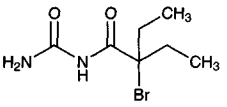
KEY WORDS

comparison with post-column reaction detection

REFERENCE

Burns,R.B.; Embree,L. Comparison of ultraviolet (UV) and post-column reaction (PC/UV) detection methods for the high-performance liquid chromatographic (HPLC) analysis of carboplatin in human plasma ultrafiltrate (PUF) (Abstract 2483), *Pharm.Res.*, **1997**, *14*, S373–S373.

Carbromal



Molecular formula: $C_7H_{13}BrN_2O_2$
Molecular weight: 237.10
CAS Registry No.: 77-65-6
Merck Index: 1879

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

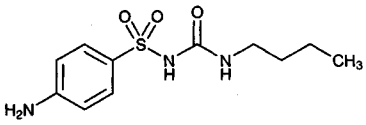
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben,

pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Carbutamide



Molecular formula: $C_{11}H_{17}N_3O_3S$
Molecular weight: 271.34
CAS Registry No.: 339-43-5
Merck Index: 1881
Lednicer No.: 1 138

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 14.547

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.